

Location of Glucose Aversion Gene on Linkage Group VIII of the German Cockroach (Dictyoptera: Blattellidae)

MARY H. ROSS¹ AND JULES SILVERMAN²

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ABSTRACT Glucose aversion in the German cockroach, *Blattella germanica* (L.) is controlled by a semidominant, autosomal gene (*Glu*). Earlier work indicated that *Glu* is linked with ruby eye (*ru*), a linkage group VIII (chromosome 9) marker. Recombination was estimated at $13.6 \pm 2.52\%$. Linkage tests with notched sternite (*st*) were undertaken to place the *Glu* locus within linkage group VIII. The results confirmed the expected linkage with *st*. Recombination was estimated at $18.2 \pm 2.1\%$, indicating that the order of loci is *Glu—ru—st*.

KEY WORDS German cockroach, genetics, behavior

AVERSION OF GERMAN cockroaches, *Blattella germanica* (L.), to D-glucose was reported by Silverman and Bieman (1993). Glucose was a component of a toxic bait to which the strain had been heavily exposed over a period of several generations. Subsequent study indicated that glucose aversion was apparently governed by a simple autosomal semidominant gene (*Glu*) and that it was present in strains from diverse geographic localities (Silverman and Ross 1994). Additional studies of *Glu* were undertaken to confirm the inheritance pattern, determine linkage relationships, and, in crosses to various mutant markers, study expression in different genetic backgrounds (Ross and Silverman 1995). *Glu* was allocated to linkage group VIII (chromosome) 9 on basis of linkage with ruby eye (*ru*). Recombination was estimated at $13.3 \pm 2.52\%$. Linkage studies with a 2nd group VIII marker, notched sternite (*st*), were undertaken to locate the *Glu* locus in the linkage group. The results are reported here.

Materials and Methods

Insects for crossing were drawn from 2 strains, the T-164 strain, kept at the Clorox Technical Center (Pleasanton, CA), and notched sternite (*st*), maintained at the Genetic Stock Center for the German Cockroach at Virginia Polytechnic Institute and State University. Selection pressure on T-164 has resulted in a strain we assume is homozygous for glucose aversion (*Glu/Glu*). Notched sternite is a recessive mutant characterized by female sterility (Ross 1966). It is maintained by re-

peatedly crossing *st/st* males to female heterozygotes (*st/+*).

Notched sternite males were mass mated to presumptive *Glu/Glu* females. Single pair matings were used in backcrosses of F₁ females to *st/st* males. Backcross progeny were scored for phenotype, either as late instars or as adults. The number of late instar or adult progeny in each cross was divided by the number of eggs in the ootheca to estimate the number of nymphs that hatched and survived throughout most or all of nymphal development.

Phenotypically wild-type and *st* backcross males from test crosses were assayed for glucose intake using the procedure described by Silverman and Bieman (1993). In brief, backcross males were offered 0.5 ml of a 2-M glucose solution prepared with 8 mM amaranth, following starvation for 48 h. This concentration gave the best discrimination between the F₁ and parent types, as well as least variation. The dye solution was left in place for 5 min, after which water was provided for 15 more minutes. Individual insects were extracted 2 times, first in ethanol:water (1:1) and next in acetone. The supernatant was measured at A520 nm and dye (glucose) intake determined by comparing absorbance reading for each insect against a calibration curve. The latter was established by feeding known volumes of 8 mM amaranth to individual cockroaches. In addition, *st* males from the mutant stock were assayed for glucose ingestion to determine whether, as expected, the stock was wild type for *Glu*. Genotypes in backcross progeny were separated by a discriminating ingestion of glucose, similarly to previous linkage tests (Ross and Silverman 1995).

Results and Discussion

Backcross progeny, scored for phenotype, fit the expected 1 +/+ : 1 *st* ratio (330 wild type; 335 *st*,

¹ Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

² Clorox Company Technical Center, P.O. Box 493, Pleasanton, CA 94566-0803.

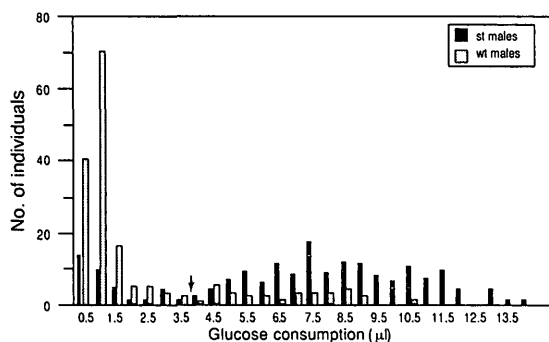


Fig. 1. Ingestion of 2 M glucose by males in backcrosses of *Glu* with *st*. Arrow indicates the discriminating ingestion ($\leq 3.5 \mu\text{l}$) used to separate *Glu* heterozygotes (*Glu*/+) from ++ ($> 3.5 \mu\text{l}$). *st*, notched sternite; wt, wild type.

$\chi^2 = 0.037$, $P > 0.80$). No deviations from the expected 1:1 sex ratio occurred in either phenotypic category. The average number of eggs per ootheca was 46.9 ± 0.91 . An average of $88.7 \pm 1.03\%$ of the nymphs hatched and survived into either the last instar or adult stage, depending on when they were counted. The percentage would have been somewhat higher if a number of *st* females among the oldest adults counted had not died. The abdomens of *st* females were distended from an accumulation of eggs. Eggs are interconnected by a complex of tubules but normal oviducts are absent. Conceivably the tubules are partial expressions of oviducts that might connect with genital papilla-like structures on nongenital segments of *st* females (Ross 1966).

In tests of 1,357 males, including 329 in the test crosses of *Glu* with *st*, the results have been remarkably consistent. In all crosses except that with yellow body-hooded pronotum double homozygotes, an ingestion of ≤ 3.5 gave a clear separation of genotypes (Ross and Silverman 1995). In the yellow body-hooded pronotum tests, a discriminating ingestion of ≤ 4.5 gave the best separation of genotypes. Fig. 1 shows backcross data from the *Glu-st* test crosses. The arrow indicates presumptive separation of *Glu*/+ and ++ (≤ 3.5 and $> 3.5 \mu\text{l}$, respectively). In males from the *st* stock, no single individual ingested $< 4.5 \mu\text{l}$ of glucose. Segregation of *Glu* in the test cross data fit the 1:1 ratio expected from monogenic segregation (169 *Glu*/+:160 ++, $\chi^2 = 0.246$, $P > 0.80$).

Segregants identified as described above were 129 *Glu*/+, 29 *Glu*/*st*, 31 ++, 140 +/*st* ($n = 329$). Recombination was estimated at $18.2 \pm 2.1\%$. Previous linkage estimates placed *ru* at 5.7 ± 2.8 map units from *st* (Ross and Cochran 1968) and *ru* at

13.6 ± 2.5 map units from *Glu* (Ross and Silverman 1995). If the *Glu* and *st* loci lie on same side of *ru*, the expected distance between *st* and *Glu* would be close to 7.9 map units; if on opposite sides, *st* and *Glu* should be separated by approximately 19.3 map units. Recombination of *Glu* with *st* agrees with the latter hypothesis. Map distances and order of loci from *Glu* linkage tests are summarized below.

Glu ----- (13.6) ----- *ru* --- (5.7) ----- *st*
Glu ----- (18.2) ----- *st*

Linkage group VIII markers not used in the work with *Glu* are tightly linked with *ru* (Ross and Keil 1978, Ross and Tanaka 1988). Therefore, the recombination estimate of *Glu* with *ru* also gives an approximation of map distances separating *Glu* from loci that lie close to *ru*, including stumpy (*sty*), miniature wing (*min*), and maxillary palp elongate (*mpe*).

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